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Amelogenin (an extracellular matrix protein) application on ischemic colon anastomosis in rats

Sıçanlarda iskemik kolon anastomozu üzerine amelogenin (bir ekstraselüler matriks proteini) uygulaması

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BACKGROUND

Ischemia is a troublesome problem that can cause intestinal emergencies and complicate the treatment. Identification of a chemical agent with beneficial effects on the healing process in risky colon anastomosis with the aim of reducing leakage rates is a popular topic in the era of surgical research. Data is lacking about the role of amelogenin, an extracellular matrix protein, during the healing process of gastrointestinal anastomosis. In this study, the effects of amelogenin treatment on ischemic colon anastomosis were evaluated.

METHODS

Adult male Wistar Albino rats weighing 200-250 g were divided into three weight-matched groups as normal colon anastomosis group (n=8), ischemic colon anastomosis group (n=8), and amelogenin-treated ischemic colon anastomosis group (n=8). Sufficient equal volume of amelogenin to cover the anastomosis area entirely was applied topically. All animals were sacrificed on postoperative day four. Bursting pressure levels were measured. Peri-anastomotic colon tissue hydroxyproline levels were also assessed.

RESULTS

Bursting pressure level of the ischemic colon anastomosis group was significantly lower than the normal colon anastomosis and the amelogenin-treated ischemic colon anastomosis groups, respectively (p=0.006, p=0.008).

CONCLUSION

Amelogenin treatment supports the physical strength of ischemic colon anastomosis.

Key Words: Amelogenin; bursting pressure; colon anastomosis; ischemia.

AMAÇ

İskemi, bağırsak kaynaklı acillere neden olan ve tedavilerini güçleştiren belalı bir sorundur. Riskli kolon anastomozlarının iyileşme sürecine olumlu katkıları olan bir yöntem veya ajan bulmak cerrahi araştırma alanında popüler bir konudur. Bir ekstraselüler matriks proteini olan amelogeninin gastrointestinal anastomozların iyileşme sürecindeki rolü ile ilgili bilgi bulunmamaktadır. Bu çalışmada, amelogeninin iskemik kolon anastomozu üzerine etkileri değerlendirilmiştir.

GEREÇ VE YÖNTEM

Yetişkin, erkek, 200-250 g ağırlığında Wistar Albino cinsi sıçanlar üç eş gruba ayrıldı: normal kolon anastomozu grubu (n=8); iskemik kolon anastomozu grubu (n=8); amelogenin uygulanan iskemik kolon anastomozu grubu (n=8). Eşit ve yeterli miktarda amelogenin anastomoz hattını tamamen örtecek şekilde topikal olarak uygulandı. Tüm sıçanlar ameliyat sonrası dördüncü günde öldürüldü. Patlama basıncı ve perianastomotik kolon dokusu hidroksiprolin seviyesi ölçüldü.

BULGULAR

İskemik kolon anastomozu patlama basıncı, normal kolon anastomozu ve amelogenin uygulanan iskemik kolon anastomozu düzeyinden anlamlı olarak düşüktü (sırasıyla p=0,006, p=0,008).

SONUÇ

Amelogenin uygulaması iskemik kolon anastomozunun fiziksel sağlamlığını destekler.

Anahtar Sözcükler: Amelogenin; patlama basıncı; kolon anastomozu; iskemi.

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Ischemia is a troublesome problem that can cause intestinal emergencies and complicate the treatment. Previous aortic surgery and chronic diseases that damage vessels and hemorrheology are the common factors that lay the groundwork for colonic ischemia.[1,2] Ischemia impairs anastomotic healing of the colon and causes cell death by depletion of the energy that is necessary for continuity of homeostasis and wound healing.[3,4] Release of vasoactive agents and oxygen-derived free radicals, depletion of high-energy phosphate molecules, inactivation of sodium and potassium pumps, edema of the endothelial cells, and thrombosis of arteries and/or veins occurring during the cascade of wound healing may cause arterial insufficiency, leading to ischemia or necrosis.[5]

In conditions necessitating the surgical treatment of colonic emergencies, some surgeons prefer primary anastomosis rather than making a temporary stoma in an effort to prevent a second surgery. This clinical reality has forced the surgical research community to identify a chemical agent with beneficial effects on the healing process of risky colon anastomosis with the aim of reducing leakage rates. Many drugs and different techniques have been evaluated with respect to their effects on the healing process in colon anastomosis.

Amelogenin is a low-molecular-weight protein found in developing tooth enamel, and it belongs to a family of extracellular matrix (ECM) proteins. Developing enamel contains about 30% protein, and 90% of this is comprised of amelogenins.[6] It has been reported that amelogenin regulates the initiation and growth of hydroxyapatite crystals during the mineralization of enamel. In addition, amelogenins appear to aid in the development of cementum by directing cells that form cementum to the root surface of teeth. Other significant proteins in enamel are ameloblastins, enamelins and tuftelins.[7] In the literature, the supportive effects of amelogenin have been reported on the healing process of oral and skin wounds.[8] These studies showed that amelogenin has beneficial effects other than in tooth tissue. However, data is lacking about the role of amelogenin during the healing process of gastrointestinal anastomosis. Thus, in this study, we evaluated the effects of amelogenin treatment on ischemic colon anastomosis.

MATERIALS AND METHODS

This study was performed after approval from the Ethics Committee of the Animal Care Review Board of Istanbul University Experimental Medicine Research Institute. Adult male Wistar Albino rats obtained from the Experimental Animal Research Laboratory of Cerrahpasa Medical Faculty, weighing 200-250 g, were used. Animals were housed in accordance with national legislation and the Council Directive of the European Communities on the Protection of Animals Used for Experimental and Other Scientific Purposes (L358/1, November 24, 1986). The rats were permitted ad libitum access to standard lab chow and tap water in cages in a regulated environment (23±2°C, 55±15% relative humidity) under a 12-hour light/dark cycle (on 8:00 to 20:00) after surgery. Twenty-four hours before surgery, the animals received only clear liquid diet. All experiments were performed in the Cerrahpasa Medical Faculty Experimental Animal Research Laboratory. The rats were divided into three weight-matched groups as normal colon anastomosis group (n=8), ischemic colon anastomosis group (n=8), and amelogenin-treated ischemic colon anastomosis group (n=8). All animals were sacrificed on postoperative day four. The biochemical parameters were evaluated in the peri-anastomotic area of the colon segment. During the preliminary studies, we applied amelogenin to normal colon anastomosis. We observed no side effects or any differences between the normal colon anastomosis and amelogenin-treated normal colon anastomosis groups. During the planning of the study, we thought that it would be more logical to apply an agent that could be beneficial during the healing process of risky intestinal anastomosis. We did not present the preliminary results to prevent probable confusion on the fluency of the article.

Surgical Procedure

We performed ischemic colon anastomosis according to the standard method, which was described previously.[3] The animals were anesthetized by intraperitoneal injection of ketamine hydrochloride (50 mg/kg of body weight). The distal colon was found through a midline abdominal incision. A 1 cm segment of left colon was resected 3 cm proximal to the peritoneal reflection in all animals. The fecal contents were milked out and a standardized end-to-end anastomosis was performed with inverting 6/0 polypropylene sutures. In the ischemic colon anastomosis groups, the whole vessels in the mesocolon between 2 cm proximal and 2 cm distal from the anastomosis line were ligated to establish ischemic colon anastomosis. The abdomen was closed with continuous sutures of 3/0 silk.

Amelogenin Application

Amelogenin was carefully reconstituted and prepared according to the manufacturer's instructions (Xelma®). Sufficient equal volume of amelogenin to cover the anastomosis area entirely was applied on the target surface using the enclosed syringe. Sufficient (0.25 ml) equal volume to cover the anastomosis area entirely was applied by topical application.

Measurement of Bursting Pressure

The abdominal incision was opened and the adhesions around the repaired area were preserved. The left

Table 1. Summary of the results with statistical significance

	Normal colon anastomosis	Ischemic colon anastomosis	Amelogenin-treated ischemic colon anastomosis
Bursting pressure levels (mm/Hg)	280±28.28	222.5±40.27† (p<0.006)	277.14±21.38‡ (p<0.008)
Hydroxyproline levels (micg/mg dry tissue)	17.21±2.86	18.5±4.07	14.93±3.4

Significant differences between the normal colon anastomosis group and the other groups defined with †.
Significant differences between ischemic colon anastomosis group and the other groups defined with ‡.

colon was ligated from the 2 cm distal part of the anastomosis area. A catheter was inserted from 2 m proximal site of the left colon and fixed with 2/0 silk fixture. The prepared system was sunk into a bowl filled with water. Air was insufflated with 6 ml/min stable speed and the bursting pressure was measured with a sphygmomanometer. The pressure level when bubbles were first observed in the water was accepted as the bursting pressure level.

Measurement of Hydroxyproline Contents

Total hydroxyproline content of the 0.5 cm-long peri-anastomotic bowel segment was measured as an assessment of bowel collagen content. A spectrophotometric assay was used to quantify bowel hydroxyproline.[9] Briefly, the bowel was removed from the -70°C freezer and homogenized in 5% trichloroacetic acid (1:9 wt/vol). The homogenized samples were centrifuged for 10 min at 4,000 g, and the pellet was washed twice with distilled water and then hydrolyzed for 16 h at 100°C in hydrochloric acid (6N HCl). The hydroxyproline level was expressed as micrograms per milligram dry tissue. All biochemical measurements were performed in a blinded fashion. All measurements were carried out in duplicate, and the average result was shown.

Statistical Analysis

The data is expressed as mean and standard deviation (mean ± SD) and 95% confidence intervals. Data were compared between groups using Kruskal-Wallis test, and Bonferroni-adjusted Mann-Whitney test was used for statistical analysis. A value of $p<0.0125$ was considered significant.

RESULTS

The results are summarized in Table 1. Bursting pressure level of the ischemic colon anastomosis group was significantly lower than in the normal colon anastomosis and the amelogenin-treated ischemic colon anastomosis groups ($p=0.006$, $p=0.008$, respectively). There were no significant differences between experimental groups with respect to hydroxyproline levels.

DISCUSSION

Anastomotic healing is affected by many local or systemic factors. Rich bacterial flora and insufficient blood supply can increase the leakage rate of colon anastomosis.[10] Various studies have evaluated the healing process of colon anastomosis in ischemic or normal conditions.[3,4,11] Ischemia is one of the most unfavorable factors negatively affecting anastomotic healing.[3] We measured the lowest bursting pressure levels in the ischemic colon anastomosis group.

Extracellular matrix (ECM) components are important factors during the healing process of colon anastomosis. The role of some ECM proteins and many factors that affect ECM components during the healing process of colon anastomosis were evaluated in different studies. As a component of ECM protein, collagen, collagen metabolism and its potential disturbance are important factors influencing the outcome of intestinal anastomotic healing.[12] It has been suggested that the ileum responds more quickly and strongly to wounding than the colon because of its high production of new collagen, and this contributes to the lower failure rate apparent for anastomoses in the small bowel.[13] Studies on the effects of synthesized ECM analogs, which have a potential as healing enhancers, on wound healing are being performed currently. Insulin-like growth factor 1, which could affect production of ECM proteins, ameliorated the adverse effects of 5-fluorouracil on the colonic healing in rats when given intraperitoneally.[14] The beneficial effects of unfractionated heparin and low-molecular-weight heparin on the healing process of colonic anastomoses in the presence of peritonitis were presented.[15] Matrix metalloproteinases, which have a role during ECM remodelling, could contribute to anastomotic dehiscence in the immediate postoperative period.[16] Positive correlations were found between treatment doses of b-human growth hormone and anastomotic defatted dry weight, hydroxyproline content and bursting strength of colonic anastomoses.[17]

It has been reported that amelogenin, which is an ECM protein, reduced ulcer size, improved the state of ulcers, reduced pain, and contributed to a larger proportion of ulcers with low levels of exudate in the treatment of venous leg ulcers and other hard-to-heal wounds.[18] We observed significantly increased bursting pressure levels in amelogenin-treated colon anastomosis in ischemic conditions. Hydroxyproline levels were similar in our experimental groups. It is known that hydroxyproline levels need not always be parallel to other parameters of wound healing.[19] Amelo-

genin treatment could mediate the healing process via a different pathway without affecting hydroxyproline content, and this process could vary under abnormal conditions such as ischemia. It has been reported that amelogenins could regulate or induce cell responses for tissue regeneration and healing, such as proliferation, migration, adhesion, and differentiation.[20]

Treatment with amelogenin, which is a novel ECM protein, supports the physical strength of ischemic colon anastomoses. This is the first study that shows the beneficial effects of amelogenin in colon anastomosis. Further studies including different biochemical parameters and techniques should be performed to define the other effects of amelogenin on intestinal anastomosis under different conditions.

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